AMENDMENTS TO THE CLAIMS

1. (Previously Presented) An *in vitro* method of determining activation or inactivation of the atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) hormonal systems in a subject, the method comprising detecting in a single reading, in a single assay the presence of atrial and brain natriuretic peptide prohormones (proANP and proBNP) or fragments thereof in a sample from the subject, wherein, compared to a reference level, detection of an increase in the presence of proANP and proBNP, or fragments thereof, in the sample indicates activation of the ANP and BNP hormonal systems, and wherein, compared to a reference level, detection of a decrease in the presence of proANP and proBNP, or fragments thereof, in the sample indicates inactivation of these systems, wherein said method does not comprise detection of the presence of proANP and proBNP or fragments thereof individually.

2. (Currently Amended) The method according to claim 1, which comprises contacting the sample with a bi- or oligo- specific first binding substance that is able to bind to:

(a) ~~(i) proANP (SEQ ID NO:1), ANP (SEQ ID NO:2), or NT-proANP (SEQ ID NO:3); or~~

~~(ii) a fragment of (a)(i) which is at least 6 amino acids in length;~~

~~(b) (i) proBNP (SEQ ID NO:4), BNP (SEQ ID NO:5), or NT-proBNP (SEQ ID NO:6); or~~

~~(ii) a fragment of (b)(i) which is at least 6 amino acids in length; and~~

~~(c) a fusion polypeptide agent or a fusion peptide agent comprising both (a) and (b);~~

~~(d)~~ proANP (SEQ ID NO:1), ANP (SEQ ID NO:2), or NT-proANP (SEQ ID NO:3); and

~~(e)~~(b) proBNP (SEQ ID NO:4), BNP (SEQ ID NO:5), or NT-proBNP (SEQ ID NO:6).

3. (Currently Amended) The method according to claim 1 which comprises contacting the sample with

- a fusion polypeptide agent ~~or a fusion peptide agent~~ comprising both:

(a) ~~(i)~~ proANP (SEQ ID NO:1), ANP (SEQ ID NO:2), or NT-proANP (SEQ ID NO:3); ~~or~~

~~(ii) a fragment of (a)(i) which is at least 6 amino acids in length;~~

and

(b) ~~(i)~~ proBNP (SEQ ID NO:4), BNP (SEQ ID NO:5), or NT-proBNP (SEQ ID NO:6); ~~or~~

~~(ii) a fragment of (b)(i) which is at least 6 amino acids in length~~;

wherein said fusion polypeptide agent ~~or said fusion peptide agent~~ can be bound by a first binding substance, and said fusion polypeptide agent ~~or said fusion peptide agent~~ is used as a calibration agent or a competitive inhibitor;

and

- said first binding substance, which is able to bind to:

(c) ~~(i)~~ proANP (SEQ ID NO:1), ANP (SEQ ID NO:2), or NT-proANP (SEQ ID NO:3); ~~or~~

~~(ii) a fragment of (c)(i) which is at least 6 amino acids in length;~~

(d) ~~(i)~~ proBNP (SEQ ID NO:4), BNP (SEQ ID NO:5), or NT-proBNP (SEQ ID NO:6); ~~or~~and

~~(ii) a fragment of (d)(i) which is at least 6 amino acids in length;~~

(e) said fusion polypeptide agent ~~or said fusion peptide agent~~;

~~(f) proANP (SEQ ID NO:1), ANP (SEQ ID NO:2), or NT-proANP (SEQ ID NO:3);~~ ~~and~~

~~(g) proBNP (SEQ ID NO:4), BNP (SEQ ID NO:5), or NT-proBNP (SEQ ID NO:6)~~.

4. (Previously Presented) The method according to claim 3 wherein the first binding substance comprises:

(a) a bi- or oligo-specific binding substance; or

(b) a mixture of mono-specific binding substances.

5 and 6. (Cancelled).

7. (Previously Presented) The method according to claim 2 wherein the first binding substance comprises an antibody or a fragment or derivative thereof.

8. (Previously Presented) The method according to claim 7 wherein the antibody comprises a polyclonal antibody, monoclonal antibody, oligoclonal antibody, bifunctional antibody or crossreacting polyclonal antibody.

9. (Currently Amended) The method according to claim 3 wherein, in the fusion polypeptide agent, (a)~~(i)~~ is SEQ ID NO:3 and (b)~~(i)~~ is SEQ ID NO: 6, or (a)~~(i)~~ is SEQ ID NO:2 and (b)~~(i)~~ is SEQ ID NO:5.

10. (Currently Amended) The method according to claim ~~3~~1 ~~wherein the fusion polypeptide agent or the fusion peptide agent comprises~~ which comprises contacting the sample with

- a fusion polypeptide agent comprising:
  - (a) $proBNP_{15-24}$ and $proANP_{82-96}$;
  - (b) $proBNP_{1-37}$ and $proANP_{29-98}$;
  - (c) $proBNP_{10-29}$ and $proANP_{20-80}$;
  - (d) $proBNP_{1-76}$ and $proANP_{1-98}$;
  - (e) $proBNP_{10-29}$ and $proANP_{60-80}$;
  - (f) $proBNP_{1-108}$ and $proANP_{1-126}$; or
  - (g) $proBNP_{77-92}$ and $proANP_{112-126}$;

  wherein said fusion polypeptide agent can be bound by a first binding substance, and said fusion polypeptide agent is used as a calibration agent or a competitive inhibitor; and
- said first binding substance, which is able to bind to:
  - (h) proANP (SEQ ID NO:1), ANP (SEQ ID NO:2), or NT-proANP (SEQ ID NO:3); and
  - (i) proBNP (SEQ ID NO:4), BNP (SEQ ID NO:5), or NT-proBNP (SEQ ID NO:6).

11. (Cancelled).

12. (Currently Amended) The method according to claim 2 wherein the first binding substance and/or the fusion polypeptide agent ~~or the fusion peptide agent~~ is:

(a) labelled with a detectable label; and/or

(b) immobilised.

13. (Previously Presented) The method according to claim 2 which additionally comprises contacting the sample with a second binding substance which is able to bind to the first binding substance.

14. (Previously Presented) The method according to claim 13 wherein the second binding substance is:

(a) labelled with a detectable label; and/or

(b) immobilised.

15. (Previously Presented) The method according to claim 13 wherein the second binding substance causes precipitation of the first binding substance and any peptide which is bound to it.

16. (Previously Presented) The method according to claim 1 which comprises an immunoassay.

17. (Previously Presented) The method according to claim 1, wherein detection of activation of the ANP and BNP hormonal systems is diagnostic of heart failure, or detection of inactivation of ANP and BNP hormonal systems monitors successful treatment of a cardiac condition.

18-28. (Cancelled).

29. (Withdrawn) A method of identifying a substance that binds specifically to

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

and

(b) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

which method comprises:

(A) contacting a candidate substance with (a) and (b) under conditions which allow specific binding; and

(B) determining whether the candidate substance binds to (a) and (b).

30. (Withdrawn) A method according to claim 29 which comprises:

contacting the candidate substance with an agent which comprises:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length; and

(B) determining whether the candidate substance binds to the agent.

31. (Withdrawn) A bi- or oligo- specific antibody, fragment or derivative thereof which is able to bind to both:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

32. (Withdrawn) An antibody, fragment or derivative according to claim 31 which is labelled with a detectable label.

33. (Withdrawn) A process for making an antibody as defined in claim 31 comprising culturing a cell that expresses the antibody and optionally purifying antibody from the cell.

34. (Withdrawn) A process according to claim 33 in which the cell is one which is obtainable by administering to a mammal, a polypeptide agent which comprises:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

extracting B cells from the mammal and selecting a cell from these based on the ability to express an antibody with the specificity such that it is able to bind both

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

35. (Withdrawn) A process according to claim 33 in which the cell is recombinant for a polynucleotide which expresses the antibody.

36. (Withdrawn) A solid support comprising an antibody according to claim 31.

37. (Withdrawn) A solid support according to claim 36 which is a particle, dipstick or microtitre plate.

38 and 39. (Cancelled).

40. (Withdrawn) A diagnostic kit comprising:

(a) a bi or oligo specific first binding substance that is able to bind to both

(I) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(II) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iv) a fragment of (i) or (ii) which is at least 6 amino acids in length; or

(b) a first binding substance and an agent as defined in claim 3;

wherein optionally the binding substance and/or the agent is labelled.

41. (Withdrawn) A kit according to claim 40 wherein the first binding substance comprises

(a) bi- or oligo-specific binding substance;

(b) a mixture of mono-specific binding substances

(c) natriuretic receptor GC-A (SEQ ID NO: 33)

(d) homologous sequence having at least 70% identity to (c)

(e) a fragment of (c) or (d) which is at least 400 amino acids in length

(f) an extracellular binding domain of the natriuretic receptor GC-A (SEQ ID NO: 34)

and/or is present on a solid support comprising a bi- or oligo- specific antibody, fragment or derivative thereof which is able to bind to both:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

42. (Withdrawn) A kit according to claim 40 wherein the agent comprises

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

43. (Withdrawn) Use of:

- a first binding substance as defined in, or
- an agent
- a polynucleotide or its complement that encodes said first binding substance or agent
- a bi or oligo specific antibody fragment or derivative thereof which is able to bind said first binding substance or agent

- a solid support which comprises said antibody; or

- a kit comprising said bi or oligo specific first binding substance and said agent

wherein said first binding substance or agent comprises:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) proBNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length

in a method for diagnosis and/or monitoring treatment of heart failure.

44. (Withdrawn) A method of diagnosing and/or monitoring treatment of heart failure in an individual comprising:

(a) obtaining a biological sample from an individual;

(b) determining the activation or inactivation of both the ANP and BNP hormonal systems in the individual by a method which comprises simultaneously detecting the presence or amount of proANP and proBNP or fragments thereof in the sample.

45-61. (Cancelled).

62. (Previously Presented) The method of claim 1, wherein said reference level is determined from a previous measurement from said subject.

63. (Previously Presented) The method of claim 1, wherein said reference level is based on the normal level of a population of subjects.

64. (Previously Presented) The method of claim 63, wherein said population of subjects is the general population.

65. (Previously Presented) The method of claim 1, wherein said assay is calibrated so that a particular reading in the assay is known to represent the normal peptide level.

66. (Previously Presented) The method of claim 1, wherein said assay is calibrated so that a normal level will produce a negligible or insignificant result.

67. (Currently amended) The method of claim 3, wherein said assay is calibrated by use of said fusion polypeptide agent ~~or said fusion peptide agent~~.

68. (Previously Presented) The method of claim 1, wherein said subject is a human.